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Chapter 5

THE ROLE OF BACTERIA IN ENVIRONMENTAL GEOCHEMISTRY

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INTRODUCTION

Many of the geochemical processes occurring at earth-surface temperature and pressure are influenced by microorganisms, particularly the bacteria. Like any other living entity, the bacteria as an aggregate have a single goal, to reproduce and perpetuate their life-form. To accomplish this goal the bacteria must utilize energy and materials that can only be obtained from their surrounding environment. As the process of growth and reproduction occurs, a variety of changes occur in the environment in which the microbe lives. The chemical makeup of waters inhabited by bacteria is altered, and any equilibrium reactions taking place in that water or between that water and associated solid phases may cause a shift in the chemical composition of aqueous- or solid-phase materials. Some materials, such as oxygen, may simply be consumed by the microbes. The resultant anoxic conditions may induce concomitant changes in some redox-sensitive species of elements such as sulfur. Bacterial utilization or production of oxidized or reduced chemical species may cause further large shifts in the redox status of the local environment. Production of acidogenic or proton-consumptive species can cause radical shifts in pH in either direction, depending on the organism, the starting material, and the products formed. Indeed, the current state of the earth's surface conditions is due in large part to the action of microorganisms on the chemicals found in the environment. Photosynthetic organisms (including the cyanobacteria, which are often referred to as blue-green algae) created the oxidizing atmosphere. Many important geochemical reactions occur against thermodynamic predictions, while others occur at rates that would be considered impossible if only the abiotic reactions operated.

The importance of the microbes (in the context of all of the life on the planet) in shaping the condition of the surface environment was summarized by Krumbein and Dyer (1985):

"1) Transfer of the rocks and minerals of the lithosphere through time and space is considerably speeded up and directed by biological processes on all scales….
2) Biological transfer of parts of objects of art and technology made of minerals, glasses and metals is considerable and by far exceeds the speed of inorganic transfer processes.
3) The transfer activity of the biosphere includes enrichments for practically all elements, purposeful arrangement according to the requirements of the biota, and rate limiting controls.
4) The abiotic transfer of rocks and minerals in a geomorphological sense is negligible under the thermodynamic conditions of earth.
5) Biotic and abiotic states of matter are in homeorhesis."

To understand how the microorganisms play such a dominating role in geochemical processes, it is necessary to look both at what the organisms do geochemically and how and why they do it. This chapter will examine the most fundamental processes of microbial growth and reproduction, and it will link those fundamental processes with the geochemical changes that are often observed to occur in the presence of microbes. It is not the purpose of this presentation to provide an exhaustive discussion; indeed, that would (and has) filled several volumes. Rather it is more appropriate to leave the reader with the firm conclusion that many of the geochemical reactions associated with the formation of some and the weathering of all ore deposits are microbiologically mediated, and that any complete study of geochemistry therefore should pay attention to the biotic components of the system being examined.

Microbes interactions with the geochemical environment occur in a variety of ways, including utilization of geochemically reactive species, production of geochemically reactive species, and alteration of the geochemical environment. Individual compounds may be altered due to direct action by microbially produced enzymes, or by the consumption or production of molecules that react with the geochemical species of interest. Alternatively, microbial action may cause a change in the geochemical environment that leads to alteration of geochemically important species in the particular system of interest.

THE ABUNDANCE AND DISTRIBUTION OF MICROORGANISMS

Although the microbiologist often has great difficulty in keeping contaminating organisms out of laboratory cultures, it was commonly assumed for a long period of time that some areas of the Earth's surface are sterile. In the past 10–20 years, that common knowledge has been demonstrated to be inaccurate. Active bacteria have been found at very high hydrostatic pressures (600 to 1,000 atmospheres) in the deep ocean. Other active bacteria have been found to exist at high temperatures (up to 350°C) in combination with elevated pressures (about 600 atm), such as the conditions found in the geothermal rift vents in the ocean floor (Baross and Deming, 1983). Even more recently, abundant, diverse, and active communities of bacteria have been found in old sedimentary deposits at depths approaching a kilometer (Balkwill, 1989). The presence of these diverse communities seems to disprove the hypothesis that microbial life is generally (but not universally) limited to the top few meters of soil (e.g., Alexander, 1976).

Given the small size of bacteria (0.5 µm to about 5 µm), one could legitimately ask the question of how they manage to be so important in the environment. The answer is quite simple: the bacteria are extraordinarily numerous, and they metabolize and reproduce very rapidly. Typical concentrations of bacteria in several habitat types are given in Table 5.1. As a means of illustration, let us assume that a person is taking a bath in “typical” lake water. If the concentration of bacterial cells is 1 x 10^6 and the bath contains 30 gallons (117 liters) of water, the bather shares the bath with 1.17 x 10^{11} other living beings! Further, given the typical numbers of bacteria, fungi, etc., recovered from surface agricultural soils, the mass of the microorganisms in an acre-furrow-slice (approximately the top 6 inches of soil covering an acre—estimated to weigh about 2 million pounds) is about 3,000 pounds.

TABLE 5.1—Typical abundance of microorganisms in various habitats.

Location	Concentration of microbes(1)	Reference
Agricultural soil	10^8 cm^{-3} (cultural counts)	Alexander, 1976
Coastal waters	10^6 ml^{-1}	Hobbie et al., 1977
Open ocean water	10^5 ml^{-1}	Atlas and Bartha, 1993
Terrestrial surface waters	10^6 ml^{-1}	Hobbie et al., 1977
Deep aquifers	10^5–10^8 g^{-1} wet sediment (cultural counts)	Balkwill, 1989
Sea floor		
Water	10^3 ml^{-1}	Deming, 1981
Sediment	10^5–10^8 g^{-1} dry sediment	Austin, 1988

(1)Numbers are approximations based on multiple values given in the reference. The numbers are given as general guidelines only; specific sites may vary from these values by several orders of magnitude.

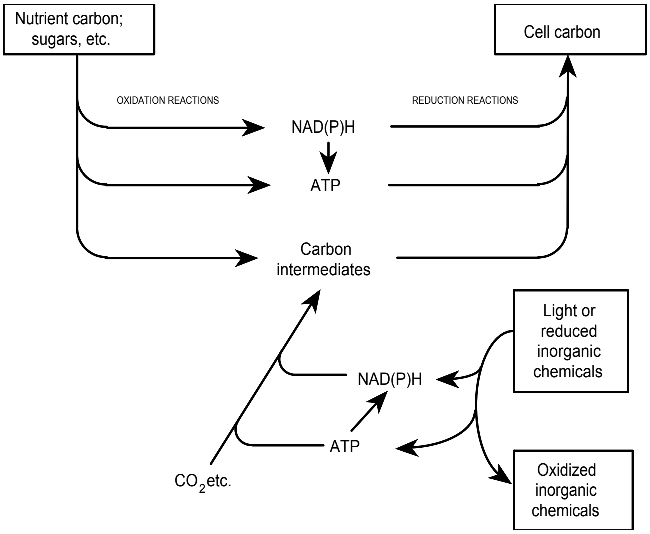

FIGURE 5.1—A summary of biological metabolism. Energy is gained from the oxidation of organic or inorganic chemicals (or light in the case of phototrophs), electrons are usually gained from those same compounds (phototrophs obtain electrons from the splitting of H_2O to yield O_2 or the splitting of H_2S to yield S°). Carbon is obtained from the organic compound used by the heterotrophs or fixed from CO_2 in the autotrophs. Note that the energy yielding (exergonic) oxidation reactions are not coupled directly to the endergonic reduction reactions of biosynthesis. Rather they are indirectly coupled through the energy transfer compound ATP and the electron transfer compound NAD(P)H. Note that NAD(P)H is a phosphorylated form of the NADH molecule mentioned in the text (after Lynch and Poole, 1979).

FUNDAMENTALS OF MICROBIAL METABOLISM

Microorganisms, as all other organisms, have several basic needs to maintain life and to grow and reproduce. Those needs can be classified by several schemes, but a fundamental one used by most microbial ecologists includes the need for energy, carbon, and a source of electrons. Along with the need for carbon goes a need for a number of other elements that are used to synthesize new biomass. For example, nitrogen, phosphorous, sulfur, potassium, calcium and several other metals and transition elements must be present in trace amounts for cell growth. Implicit in the need for a source of electrons is the need for a suitable terminal electron acceptor, a “place to put the electrons” after they have been used for energy production or reducing power.

With the exception of the phototrophs, which obtain their energy from light, all organisms are chemotrophs. That is, their energy for growth is obtained through the oxidation of either organic or inorganic chemicals (Fig. 5.1); the free energy change associated with oxidation reactions is usually negative, thus favorable for the progress of the reaction and thus resulting in a net release of energy that can be used by the organism. Life forms from microbes to man have evolved the capability of coupling the energy released from oxidation reactions to other reactions that result in the synthesis of new cellular biomass. Biosynthetic reactions are typically reductive in nature, and require an input of energy and, by definition, electrons. The exergonic oxidations are not coupled directly to the endergonic synthetic reactions. Instead, the free energy and the released electrons are held in carrier molecules. Energy is stored in the form of high-energy phosphate bonds in phosphorylated adenylate molecules (adenosine triphosphate, or ATP) and the electrons are transferred by electron carriers such as nicotinamide adenine dinucleotide (the reduced form is noted as NADH, the oxidized form as NAD$^+$). The ability to store energy and electrons in carrier molecules means that reactions with small changes in free energy can fuel reactions with larger changes in free energy without direct stoichiometric relationships.

Organisms that obtain carbon from an organic molecule are referred to as heterotrophs, whereas organisms that must reduce CO_2 to an organic form are called autotrophs (see Table 5.2). Heterotrophs most often utilize the same organic molecule as a source of energy, carbon, and electrons. While such organisms are technically chemoorganoheterotrophs, they are usually referred to simply as heterotrophs or conventional heterotrophs (Brock et al., 1979). In most (but certainly not all) chemolithotrophs, the source of energy and electrons is the same inorganic compound, while CO_2 serves as the carbon source. Thus, the two most frequently encountered nutritional types are the heterotrophs and the chemolithotrophs. In geochemically interesting systems such as ore deposits, these two broad groups are most often responsible for microbiological reactions of importance.

Chemolithotrophs are responsible for the oxidation of a variety of inorganic compounds including molecular hydrogen, reduced-sulfur and nitrogen compounds, iron(II), and even carbon monoxide (Table 5.3). The net reactions mediated by the chemolithotrophs are all favored thermodynamically, that is $\Delta G°'$ is negative. Due to kinetic constraints, however, most of these reactions proceed abiotically very slowly or not at all at earth surface temperature and pressure; a notable exception may be the oxidation of Fe^{2+}, as discussed later.

TABLE 5.2—Trophic grouping of microorganisms.

Substance	Source	Classification
Carbon	Organic	Heterotroph
	CO_2	Autotroph
Energy	Light	Phototroph
	Chemical	Chemotroph
Electrons	Organic	Organotroph
	Inorganic	Lithotroph

TABLE 5.3—Physiological groups of chemolithotrophs (taken from Gottschalk, 1986). Although the correct SI units for these values would be Joules, the author defers to the argument of Kleiber (1972) and Battley (1987), that the calorie is a preferable means of expressing changes in heat due to work done on or by a thermodynamic system.

Group	ATP yielding reaction	$\Delta G^{\circ\prime}$ kcal/reaction	kcal/2e^-
Hydrogen bacteria	$H_2 + {}^1/_2O_2 \rightarrow H_2O$	-56.7	-56.7
Carboxydo-bacteria	$CO + {}^1/_2O_2 \rightarrow CO_2$	-61.5	-61.5
Sulfur bacteria	$S^{2-} + 2O_2 \rightarrow SO_4^{2-}$	-189.9	-47.5
	$S^o + {}^1/_2O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$	-139.8	-46.6
Iron bacteria	$Fe^{2+} + {}^1/_4O_2 + H^+ \rightarrow Fe^{3+} + {}^1/_2H_2O$	-10.6[a]	-21.2[a]
Ammonia oxidizers	$NH_4^+ + 1{}^1/_2O_2 \rightarrow NO_2^- + 2H^+ + H_2O$	-64.7	-21.6
Nitrite oxidizers	$NO_2^- + {}^1/_2O_2 \rightarrow NO_3^-$	-18.5	-18.5

[a] $\Delta G^{\circ\prime}$ values for pH = 0 are given; iron bacteria can grow at acidic pH values only; the $\Delta G^{\circ\prime}$ value for pH = 7 would be -0.95 kcal/reaction.

Oxidation of a number of inorganic substances can support chemolithotrophs, and the potential (E_o’) for some of these reactions is shown in Table 5.4 along with the NADH/NAD+ couple. These reactions usually occur under aerobic conditions, because of the high E_o’ of the H_2O/O_2 couple.

TABLE 5.4—Redox potentials of reactions important in chemolithotrophic metabolism.

	E_o’ (volts)
$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$	-0.54
$H_2 \rightarrow 2H^+ + 2e^-$	-0.41
$NADH + H^+ \rightarrow NAD^+ + 2e^- + 2H^+$	-0.32
$H_2S \rightarrow S + 2H^+ + 2e^-$	-0.25
$S + 3H_2O \rightarrow SO_3^{2-} + 6H^+ + 4e^-$	+0.05
$SO_3^{2-} + H_2O \rightarrow SO_4 + 2H^+ + 2e^-$	-0.28
$NH_4^+ + H_2O \rightarrow NO_2^- + 8H^+ + 6e^-$	+0.44
$NO_2^- + H_2O \rightarrow NO_3^- + H^+ + 2e^-$	+0.42
$Fe^{2+} \rightarrow Fe^{3+} + e^-$	+0.78
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	+0.86

Under anaerobic conditions, heterotrophs often oxidize organic carbon to CO_2 by using some oxidized inorganic (other than O_2) as the terminal electron acceptor. Such organisms include the denitrifiers (NO_3^- is reduced sequentially to NO_2^- then to N_2), iron reducers, sulfate reducers, and methanogens. The latter often follow a metabolic path wherein H_2 is oxidized to H_2O, and CO_2 serves as both the carbon source and as the terminal electron acceptor (i.e., $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$). Some relevant reduction reactions will be discussed below. However, it is important to note that the chemolithotrophic oxidation of reduced inorganic species, coupled with the anaerobic reduction of the oxidized species of the same element, constitutes a complete biogeochemical cycle, at least in terms of production and fate of the various redox species.

DIRECT TRANSFORMATIONS: ALTERATIONS OF MATERIALS FOUND IN ORE DEPOSITS

Sulfur

Many of the ore deposits in which microorganisms play an important role during weathering contain reduced sulfides. The sulfides were formed as part of igneous or hydrothermal processes. Sulfides of biogenic origin are present in some mineral deposits and coal deposits. In such circumstances, the sulfides arose from the bacterial reduction of sulfate during anaerobic oxidation of organic matter. Sulfides in mineral deposits, coal deposits, and rocks are preserved until exposed by erosion to oxygen and water. A number of metal sulfides are of interest (either because they can be formed biogenically or are decomposed biologically); the reader is referred to Alpers and Nordstrom (1999) for a list of important sulfide minerals.

Sulfur oxidation

Microorganisms attack metal sulfides directly or indirectly, resulting in mineral dissolution or the combined dissolution of the mineral and the oxidation of one or more of the elements in the mineral. In the direct case, enzymes are produced by the microbes that react with the surface of the mineral grains. All studies conducted thus far suggest that close contact between the microbial cell and the mineral grain must occur for the direct dissolution/oxidation to proceed.

Metal sulfides are acted upon directly by a few microbes, generally of the genus *Thiobacillus*. Nearly all the sulfur oxidizers are chemolithoautotrophs that are capable of linking the free energy released during the oxidation with ATP and NADH formation to synthesize new cell material from autotrophically fixed CO_2. While *Thiobacillus* sp. are the primary oxidizers of the metal sulfide ores, a few genera (including some *Thiobacillus* species) attack the sulfur in dissolved sulfides (H_2S); for example, much of the H_2S formed during sulfate reduction in salt marshes is reoxidized to S^O by *Beggiatoa*. The S^O is stored as granules in the cells; in the event that the H_2S supply becomes limited, the organism is capable of oxidizing the S^O to SO_4^{2-}. In the case of *Beggiatoa*, the acidification normally seen in the oxidation of sulfide minerals such as pyrite does not occur.

In reactions involving iron-containing sulfides, it is difficult to separate the oxidation of sulfur from that of iron, and some mention of both must be made when discussing either sulfur oxidation or iron oxidation. Typical chemical reaction for oxidation of pyrite sulfur is shown in equation [1], sulfur reduction in equations [2–4], and Fe oxidation in equations [5–8]. The oxidation of pyrite proceeds according to the reaction:

$$FeS_2 + 3^1/_2O_2 + H_2O \rightarrow Fe^{2+} + 2H^+ + 2SO_4^{2-} \qquad [1]$$

The ferrous iron generated by this process can be further oxidized to ferric iron (see equation [5]). Ferric iron is then free to participate in further "indirect" oxidation according to a reaction of the type given in equation [8]. The process depicted in equation [1] is carried out by *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, possibly other members of the genus *Thiobacillus*, and members of the genus *Sulfolobus* (Erlich, 1995). The reaction depicted in equation [1] is the rate limiting step in pyrite oxidation (Singer and Stumm, 1970), and while it will proceed in the absence of bacterial catalysis, the rates in the presence of active bacteria can be as much as a million times more rapid than those in the absence of bacterial activity.

T. ferrooxidans is the most intensively studied of the sulfur-oxidizing chemolitho-autotrophs. In addition to its oxidation of sulfur, *T. ferrooxidans* can also oxidize ferrous iron in pyrite and other sulfide minerals to drive its biosynthetic pathways. Indeed, to separate for laboratory studies *T. ferrooxidans* from *T. thiooxidans*, which can oxidize the sulfur but not the iron, a medium containing reduced iron added as $FeSO_4$ (the so-called 9K medium of Silverman and Lundgren, 1959) is used to grow the iron oxidizers. *T. ferrooxidans* cannot use ferrous iron at a pH above 4. There, the free energy change of the reaction becomes too small to allow efficient ATP synthesis for energy storage (see Table 5.3). *T. ferrooxidans* can oxidize sulfur at circumneutral pH levels, however.

Equation [1] shows the production of one mole of H^+ per mole of pyrite sulfur oxidized. This reaction, therefore, contributes to a number of acidification processes, including those that form acid mine drainage (AMD). As we shall see below, the oxidation of Fe^{2+} contributes even more acid to the water in which the reaction is taking place.

Sulfate reduction

In the absence of oxygen, a select but active and important group of microbes are capable of oxidizing organic matter while utilizing SO_4^{2-} as the terminal electron acceptor. This reaction is often written as

$$2(CH_2O) + SO_4^{2-} \rightarrow S^{2-} + 2H_2O + 2CO_2 \qquad [2]$$

where CH_2O is used to represent organic matter (CH_2O is a good stoichiometric representation of carbohydrate, the most abundant material in plants). Work of the past 10 years has shown that equation [2] represents a community-level reaction in that organisms that actually reduce SO_4^{2-} (typified by members of the genus *Desulfovibrio*) cannot use carbohydrates. Rather they utilize the products of anaerobic bacterial fermentation such as acetate and H_2, the most frequently used substrates for the direct reduction of SO_4^{2-} to H_2S.

Sulfate reduction is an important reaction in the geochemical processes associated with the formation of many sulfides in coal and some mineral deposit types. It represents the reverse of one of the important reactions that creates the acidic conditions associated with the oxidation of sulfide minerals. Indeed, sulfate reduction represents the source of sulfide in most coal deposits; seawater sulfate is reduced during the initial decomposition reactions associated with coal formation. Pyrite forms as a result of the simultaneous release of Fe^{2+}.

In addition to being the source of reduced sulfide in coal and some mineral deposits, sulfate reduction can have a definite mitigative effect on the acid conditions produced by oxidation of metal sulfides such as pyrite. The fate of the sulfide released by the reaction depicted in equation [2] is likely to be as follows (Berner et al., 1970; Goldhaber and Kaplan, 1975):

$$S^{2-} + 2CO_2 + H_2O \rightarrow H_2S + 2HCO_3^- \qquad [3]$$

Combining equations [2] and [3] yields

$$2(CH_2O) + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \qquad [4]$$

The alkalinity thus produced is permanent only if the H_2S is removed (by degassing) or if the sulfide reacts with a metal ion such as Fe^{2+} to form a solid phase that prevents diffusion of the sulfide to oxygenated waters. Formation of alkalinity in acidic wastes from ore deposits will be considered further in discussion of the reduction of iron below.

Iron

Iron oxidation

As pointed out above, microbial pyrite oxidation by *T. thiooxidans* and *T. ferrooxidans* results in the formation of sulfuric acid (equation [1]). Additionally, *T. ferrooxidans* oxidizes the Fe^{2+} to Fe^{3+} by the reaction:

$$2\ Fe^{2+} + {}^1/_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \qquad [5]$$

This reaction would seem to consume the protons released during the oxidation of the sulfur, but hydrolysis of the Fe^{3+} generates a net increase in the proton concentration of the system by the formation of aqueous ferric hydroxide complexes or ferric hydroxide solids, by the reaction:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \qquad [6]$$

The formation of $Fe(OH)_3$ solids is responsible for the reddish to yellowish staining of rocks commonly seen in acid mine streams. The formation of jarosite in sulfate-rich acid mine waters may decrease the ratio of protons generated per iron oxidized (Erlich, 1995):

$$M^+ + 3Fe(OH)_3 + 2SO_4^{2-} \rightarrow MFe_3(SO_4)_2(OH)_6 + 3OH^- \qquad [7]$$

where M^+ may be Na^+, K^+, NH_4^+, or H_3O^+. The ferric iron produced may also contribute to the abiotic oxidation of pyrite with a substantial net increase in the total acid production (Erlich, 1995):

$$FeS_2 + 14\ Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad [8]$$

In this case, the dissolved ferric iron comes largely as a result of bacterial oxidation. The formation of the large amount of acidity contributes greatly to the oxidation of other metal sulfides that may be a part of the host rock in which the minerals exist.

Iron reduction

Reduction of iron (and perhaps other metals) plays an important role in ion mobility due to the increased solubility of Fe(II) compounds compared to Fe(III) compounds. Increased iron concentrations in ground waters are often attributed to the action of microorganisms, either by direct reduction of the iron when it is used as a terminal electron acceptor for oxidation of organic matter, or by indirect iron reduction caused by production of suitable reducing agents by bacteria. For example, the reduction of sulfate results in production of sulfides that can reduce Fe(III) in many minerals in the absence of direct microbial activity on the iron itself. In the case of sulfides, however, the end product may be an iron mono- or di-sulfide solid phase, thereby creating another insoluble mineral phase. Some studies of direct iron reduction by bacteria suggest that the role of this process in nature may have been underestimated historically (Lovely, 1991).

The formation of acidic effluents associated with reduced iron minerals exposed to oxygenated water relies to a great extent on the hydrolysis of the oxidized iron as shown in equations [6] and [8]. Conversely, if the iron is again reduced, consumption of that acidity occurs. This process is of particular interest in iron- and sulfur-rich acid mine waters. Given anaerobic conditions and appropriate bacteria, alkalinity can be produced from the oxidation of glucose by the community-level reaction (Mills et al., 1989):

$$3Fe(OH)_{3(s)} + 6(CH_2O) \rightarrow CH_3CH_2OCOO^- + CH_3COO^-$$
$$+ CO_{2(g)} + 3Fe^{2+} + 4OH^- + 0.5H_{2(g)} + 4H_2O \qquad [9]$$

Likewise, alkalinity is generated if lactate is used as a carbon and electron source for iron reduction, and the stoichiometry of the equations suggests that more alkalinity is generated per mole of iron reduced when lactate is used than when glucose is used:

$$Fe(OH)_3(s) + CH_3CH_2OCOO^- \rightarrow CH_3COO^- + CO_{2(g)}$$
$$+ Fe^{2+} + 2OH^- + 1.5H_{2(g)} \qquad [10]$$

Alkalinity is also generated by sulfate reduction using lactate as a carbon source:

$$SO_4^{2-} + CH_0CH_2OCOO^- + H_2(g) \rightarrow CH_3COO^-$$
$$+ CO_{2(g)} + 2OH^- + H_2S_{(g)} + H_2O \qquad [11]$$

In order for alkalinity generated during iron or sulfate reduction to be permanent, the reduced iron and sulfur must be either sequestered in, or removed from the sediments. Sequestration can occur by reactions of sulfide with metal cations to form insoluble metal sulfides and by reactions of sulfide to form organic sulfide compounds (Anderson and Schiff, 1987).

The reactions above indicate that iron reduction can be important in acid neutralization. In addition to direct acid neutralization, iron can also play a role in the delivery of SO_4^{2-} to anaerobic sites such as sediments, as well as influencing the final products of other microbial processes such as SO_4^{2-} reduction. Figure 5.2 shows a series of processes that have been shown to function in the Contrary Creek arm of Lake Anna in Virginia. Microcosm experiments utilizing sediment and water from this acid-mine-drainage-receiving impoundment have shown that removal of SO_4^{2-} from the water column is greatly enhanced by the addition of Fe^{3+} to sulfate-rich water overlying reducing sediment (Mills et al., 1989; Herlihy and Mills, 1989). The iron generates an iron oxyhydroxide floc that coprecipitates with adsorbed sulfate, enhancing delivery of SO_4^{2-} to the sediment; in contrast, direct diffusion of SO_4^{2-} into the sediments in Lake Anna accounted for only 5% of the observed sulfate retention in the acidified arm of the impoundment (Herlihy et al., 1987). The precipitation of the iron increases the acidity, but the acidity is neutralized by subsequent reactions in the sediment.

When the precipitate reaches the sediment/water interface, the iron oxyhydroxide dissolves as iron is reduced, both by direct bacterial activity and by redox reactions induced by microbial reactions not directly involving iron (Bell et al., 1987; Lovely, 1991; Lovely and Phillips, 1988). Reduction of iron neutralizes the acidity formed during the hydrolysis, and liberates adsorbed and precipitated SO_4^{2-} into the anaerobic sediments. At this point, reduc-

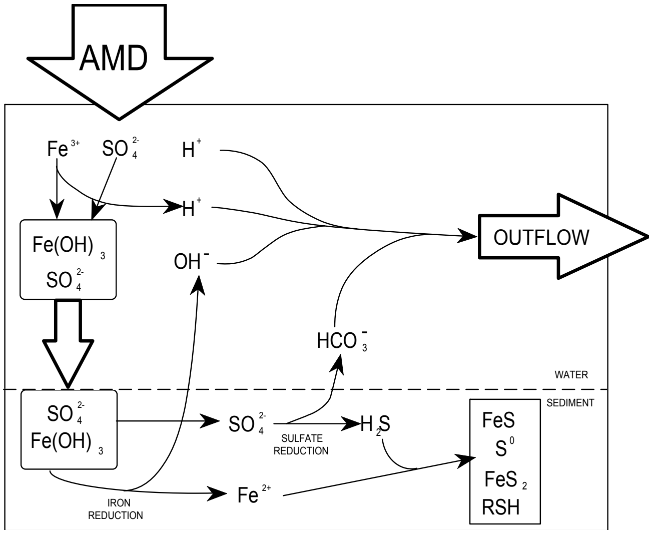

FIGURE 5.2—Role of iron and sulfate in the neutralization of acid mine drainage.

tion of both iron and SO_4^{2-} contributes to the neutralization of the acid mine drainage. While much of the neutralization contributed by iron reduction balances the acidity formed during the hydrolysis, any particulate ferric iron entering the lake can contribute to neutralization of the protons in the acid pollution.

In order to preserve the alkalinity generated by the reduction reactions the sulfides must be retained (as described above), or their reoxidation will generate protons and consume the alkalinity. Precipitation of reduced metal sulfides serves as an important mechanism for preservation of reduced sulfide. Thus, iron is a critical element in the biogeochemical neutralization of acid pollutants. In waters where iron is not abundant, other elements may also play an important role, i.e., aluminum oxides might help remove sulfate from water as would any solid-phase anion exchange complex; reduction of elements such as manganese can also contribute to the generation of alkalinity in some waters. While iron is not well studied with respect to reactions such as those described here, the role of the other elements is totally undocumented and also deserves attention to arrive at a complete picture of the overall neutralization process.

Other elements

Release of other metal ions to solution occurs during oxidation of sulfide ores either by acid production or dissolution of the solid phase. For example, the oxidation of CuS by *T. ferrooxidans* proceeds because of the oxidation of the S atom:

$$CuS + 1/2O_2 + 2H^+ \rightarrow Cu^{2+} + S^o + H_2O \qquad [12]$$

An additional, bacterially mediated step results in the formation of sulfate:

$$S^o + 1\,1/2O_2 + H_2O \rightarrow 2H^+ + 2SO_4^{2-} \qquad [13]$$

In some cases, such as that with *Thiobacillus thioparus*, the initial oxidation (equation [12]) is abiotic, and the only biotic oxidation is that depicted in equation [13] (Erlich, 1995).

Erlich (1995) also provides evidence from the literature that suggests different oxidation mechanisms for different ores. For example, the oxidation of chalcopyrite probably proceeds by a pair of reactions, the first of which is bacterially mediated, and in which both the metal and sulfide moieties are attacked by different enzymes:

$$4CuFeS_2 + 17O_2 + 4H^+ \rightarrow 4Cu^{2+} + 4Fe^{3+} + 8SO_4^{2-} + 2H_2O \qquad [14]$$

$$4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_3 + 12H^+ \qquad [15]$$

Summing reactions 14 and 15 yields:

$$4CuFeS_2 + 17O_2 + 10H_2O \rightarrow 4Cu^{2+} + 4Fe(OH)_3 + 8SO_4^{2-} + 8H^+ \qquad [16]$$

Alternatively, bacteria may attack the metal before the sulfide. Chalcocite oxidation proceeds as described by Erlich (1995):

$$Cu_2S + 1/2O_2 + 2\,H^+ \rightarrow Cu^{2+} + CuS + H_2O \qquad [17]$$

$$CuS + 1/2O_2 + 2H^+ \rightarrow Cu^{2+} + S^o + H_2O \qquad [18]$$

$$S^o + 1\,1/2O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-} \qquad [19]$$

In this case it is not clear that energy or electrons needed for growth are actually obtained by the microorganism.

Similarly, in the case of reductions, it is not clear if the redox reaction is carried out actively by the microbes or whether it occurs secondarily as a result of microbial alteration of the environment. Note, however that there appears to be strong evidence for a mercury reductase system in several bacteria (Wood, 1974). The product of mercury reduction is elemental mercury, which readily volatilizes into the atmosphere.

Several elements (notably Hg, Sn, As, Se) are known to be methylated in anaerobic sediments to volatile methyl complexes such as mono- and dimethyl mercury. Because these compounds are vaporous, they diffuse rapidly through the water, and can even diffuse into the atmosphere. The volatilization/methylation reactions are thought to be part of a strategy on the part of the microbes to convert the toxic material to a form that rapidly dissipates from the vicinity of the cell. For these reactions, microorganisms and organic matter are essential. Methylation often occurs in environments conducive to methanogenesis (i.e., sediments and anaerobic waters), and all of the known organisms that carry out such reactions are heterotrophs (both bacteria and fungi are capable of participating in such reactions).

INDIRECT TRANSFORMATIONS: ALTERATIONS OF THE GEOCHEMICAL SETTING

Alteration of pH

The reactions surrounding the oxidation of metal sulfide ores generate large amounts of acidity (See equations [1], [5], [6], and [8], above). However, if a major component of the ore body consists of a nonsulfidic, reduced iron mineral, the lack of sulfide oxidation does not preclude acid formation.

Bacteria can also reduce the pH through the formation of organic acids. These acids usually are carboxylic acids (i.e., R-COOH). Although phenols (hydroxybenzene) can also display an acid character at high pH values. Such compounds are Lowry-Brønsted acids in that they donate protons to the solution. These acids and their conjugate bases can provide buffer capacity to an aqueous solution, and the conjugate bases (e.g., $R\text{-}COO^-$) often complex with metal cations as described later.

Under some conditions, microbes can raise the pH through generation of bicarbonate alkalinity or generation of bases such as NH_3 or S^{2-}.

Alteration of Eh

Whenever microbial activity occurs, the effect is a lowering of the redox potential. In aerobic systems, the presence of oxygen poises the Eh so that visible effects are unlikely. When the oxygen is consumed, however, the measured or calculated redox potential can drop very quickly. Theoretical calculations of Eh using couples assumed to dominate under different sets of metabolic conditions indicate that the range of Eh conditions inhabited by bacteria ranges from about 800 mV (fully aerobic conditions) to about -350 to -400 mV, where methanogenesis occurs. Organic loading of any water can alter the Eh of the water if the oxygen demand exceeds the reaeration capability. The redox reactions that occur may cause the release of iron, manganese, (Evans et al., 1977; Graybeal and Heath, 1984; Brannon et al., 1985) and arsenic (Edenborn et al., 1986) to the water. Further, metals that are sorbed or precipitated with Fe and Mn oxides may also be released on dissolution of the oxide phases (Graybeal and Heath, 1984; Sawlen and Murray, 1983; Gunkel and Sztraka, 1986; Shaw et al., 1990). Recent laboratory experiments indicate the release of Cd, Cr, Ni, Pb, and Zn by this process (Francis and Dodge, 1990).

Production of chelators and complexing agents

An important microbial activity that can mobilize metals in waters associated with ore deposits is the formation of chelating agents. For example, there are a number of compounds that are used by bacteria for the transport of iron across the cell membrane. These compounds are called siderophores, and some examples of their structures are shown on Figure 5.3. Production of similar compounds for other metals is not well documented, although generation of some nonspecific complexing agents referred to as metallothioneins is frequently reported. Metallothioneins are small proteinaceous molecules with a high proportion of sulfur-containing amino acids that complex strongly with metal ions.

Microbial decomposition of organic matter often results in the release of metal-mobilizing humic substances into solution (Reuter and Perdue, 1977; Bovendeur et al., 1982). These substances contain a high concentration of acid functional groups that carry a negative charge at most environmental pH values. Thus, they tend to form strong ionic complexes, often multidentate, with dissolved metals. The affinity of the humic acids for metal ions is often strong enough to remove the ion from exchange surfaces in the solid phase of subaqueous environments. Lehman (1991) demonstrated that addition of humic acid to chambers placed on the sediment surface in Lake Anna, where the sediments are copper-enriched due to the inflow of acid mine drainage, resulted in the mobilization of copper at levels beyond that explained by mechanisms such as Eh change or reductive dissolution of copper-containing iron oxides (See Fig. 5.4)

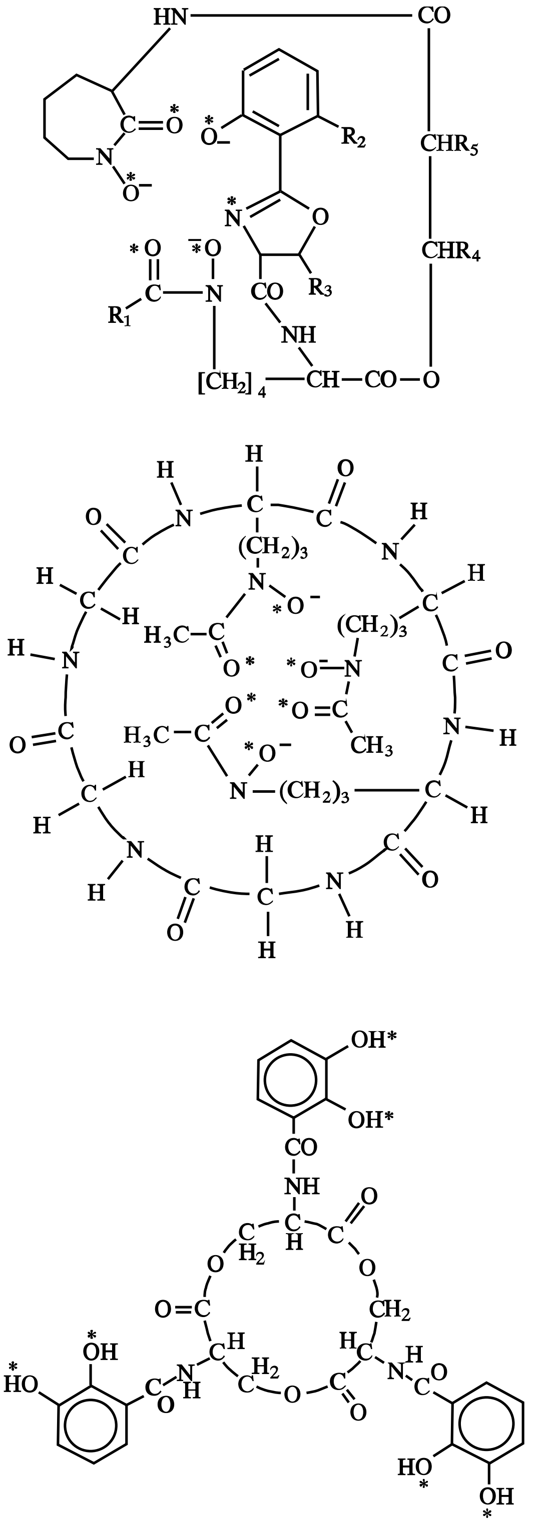

FIGURE 5.3—Structure of some siderophores. Note the variety of charge sites that can interact with a metal ion to form a multi-dentate complex.

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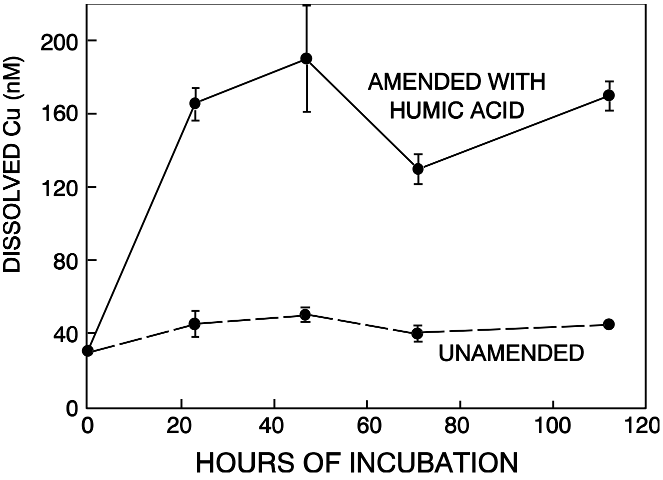

FIGURE 5.4—Release of copper from impoundment sediments due to the addition of humic acid to the overlying water. Addition of organic matter as dead (previously frozen) phytoplankton did not have the same effect on copper mobilization as did the addition of the humic acids (Lehman, 1991).

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